Stimuli-Responsive Multilayered Hybrid Nanoparticle/Polyelectrolyte Capsules


Introduction

Nanoparticles have become the subject of intensive research during the past decade. Their use in different fields such as optoelectronics,[1] catalysis,[2] diagnostics,[3] drug delivery,[4,5] etc. has stimulated scientists in different field, such as physics, chemistry, biotechnology, etc. to focus their research on these types of materials. Gold nanoparticles (AuNP) have shown high potential as their physical properties can easily be linked to inter-particle interactions.[6] Therefore, the incorporation of these nanoparticles into supramolecular structures is of great interest to a broad field as these supramolecular structures are easier to handle and allow adding supplemental functionalities. The layer-by-layer (LbL) technique is a highly versatile methodology to fabricate multifunctional supramolecular entities with tunable properties.[7] This LbL technique is based on the sequential adsorption of charged species onto an oppositely charged surface. By

Gold nanoparticles (AuNP) with carboxyl groups on their surface were used in combination with PAH for the layer-by-layer coating of CaCO3 microparticles, followed by the dissolution of the CaCO3 core. SEM, TEM, and confocal microscopy are used to characterize the hybrid nanoparticles/polyelectrolyte capsules. As the AuNP have carboxyl groups on their surface, their charge density is pH dependent; therefore, the capsules exhibit a pH-dependent swelling and can be deconstructed both at low and high pH. By covalent cross-linking of the carboxyl groups of the AuNP and the amino groups of the PAH, it is possible to suppress the pH-responsive behavior. AuNP are used as activation centers using IR light and this ability is used to release encapsulated material from the nanoparticles/polyelectrolyte capsules as well as for the enhancement of detection and imaging of such capsules by Raman microspectroscopy.
consecutive adsorption steps one is able to build up multilayers which are stabilized through electrostatic interactions. Recently, this technique has been applied for the fabrication of tiny three-dimensional microstructures, called hollow polyelectrolyte capsules, by LbL coating of a sacrificial template followed by the dissolution of this template.

Due to the multifunctionality of the LbL technique, it is possible to fabricate tailor-made capsules with functionalized or stimuli-responsive walls which could be promising for drug-delivery applications. Nanoparticle-containing capsules have already been reported in literature, although in all cases the number of nanoparticle layers was limited to one or the nanoparticles were introduced (e.g., by synthesis within the capsule wall) after the fabrication of the capsules. However, multilayered capsules consisting of alternating nanoparticle/polyelectrolyte layers have not yet been reported before.

In this paper, we report on the fabrication of pH-sensitive AuNP with a size ranging from 1 to 5 nm and use these nanoparticles for the fabrication of truly hybrid FITC-dextran filled microcapsules which consist from alternating gold nanoparticle/poly(allylamine hydrochloride) (PAH) multilayers. The structure of the microcapsules is investigated with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As both the AuNP as well as PAH have a pH-dependent charge density, the obtained capsules are pH responsive and the behavior of the capsules is studied in alkaline, neutral, and acidic environment. Furthermore, as AuNP are able to absorb light, we show that by irradiation of the capsules at high laser intensity it is possible to destroy the capsules and release their encapsulated material. IR-laser sensitive capsules could have potential as drug delivery system, e.g., for remote release after subcutaneous injection of capsules. On the other hand, IR-laser light at lower intensities can be used to visualize the capsules by Raman microscopy.

**Materials and Methods**

**Materials**

PAH (Mw ≈ 70 kDa), FITC-dextran (Mw ≈ 2 000 kDa), hydrogel tetrachloroaurate trihydrate (HAuCl₄·3H₂O), mercaptosuccinic acid (MSA), and sodium borohydride (NaBH₄) were purchased from Sigma-Aldrich-Fluka. Methanol, sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), calcium chloride (CaCl₂), and sodium carbonate (Na₂CO₃) were purchased from Merck. Quartz slides were purchased from Hellma Optics. All water used in the experiments was of Milli-Q grade.

**Synthesis of Gold Nanoparticles (AuNP)**

HAuCl₄·3H₂O (425 mg) (1.25 × 10⁻³ mol) was dissolved in 5 mL of water. 188 mg (1.25 × 10⁻³ mol) MSA was dissolved in 245 mL of methanol. Both solutions were mixed and stirred for 30 min at room temperature. 473 mg of (12.5 × 10⁻³ mol) NaBH₄ was dissolved in 25 mL of water and dropwise added to the HAuCl₄·3H₂O/MSA mixture under vigorous stirring. During the addition of the NaBH₄, the solutions gradually turned dark and the AuNP were formed as a dark precipitate. The reaction mixture is further stirred for 1 h at room temperature. Afterwards, the reaction mixture was centrifuged at 4 000 rpm for 5 min and the supernatant was discarded. Three washing centrifugation steps with methanol were performed and finally the AuNP were dried under vacuum.

**Fabrication of CaCO₃ Microparticles**

CaCO₃ particles were fabricated according to Volodkin et al. Briefly, CaCl₂ and Na₂CO₃ solutions (0.33 M) were mixed under vigorous stirring for 30 s leading to the precipitation of CaCO₃ particles. Subsequently, four centrifugation and washing steps with pure water were performed in order to remove the unreacted species. In the last step, the particles were washed with acetone and subsequently air-dried. Fluorescein isothiocyanate (FITC) dextrans were incorporated in the CaCO₃ particles by coprecipitation. Therefore, 5 mg of FITC-dextran was dissolved in the CaCl₂ solution (3.5 mL) before mixing with the Na₂CO₃ solution.

**UV-Vis Monitoring of the Multilayer Build-up**

Quartz slides were cleaned and hydrophilized by treatment with freshly prepared piranha solution (35% H₂O₂/98% H₂SO₄ 1:1 v/v; caution: piranha solution reacts violently with organic materials and should not be stored in closed containers) then abundantly rinsed with distilled water. The treated quartz slides were precoated with a PEI layer by immersion in a 2 mg·mL⁻¹ PEI solution containing 0.5 M NaCl. Multilayers of AuNP/PAH were deposited on the PEI-coated quartz slides starting with the negatively charged AuNP by immersion in a 1 mg·mL⁻¹ AuNP solution (containing 0.5 M NaCl) for 10 min followed by rinsing with pure water. Finally the quartz slides were dried under nitrogen stream. Then the PAH layer was deposited from a 2 mg·mL⁻¹ PAH solution (containing 0.5 M NaCl). This was repeated until the desired amount of bilayers was deposited. After each deposition of a bilayer the absorbance of the film was measured with a Pharmacia Biochrom 4060 UV-vis spectrophotometer.
Fabrication of Multilayered Capsules

Capsules were fabricated in a two-step procedure. In the first step, the CaCO₃ microparticles were coated using the LbL technique. Therefore, 20 mg of FITC-dextran containing CaCO₃ microparticles were dispersed in a 0.5 M NaCl solution containing the AuNP (1 mg·mL⁻¹). The dispersion was continuously shaken for 10 min. Excess of AuNP was removed by three centrifugation-washing steps with deionized water. Thereafter, 1 mL of a 0.5 M NaCl solution containing PAH (2 mg·mL⁻¹) was added and the dispersion was continuously shaken for 10 min followed again by three centrifugation-washing steps. This procedure was repeated four times until four AuNP/PAH bilayers were deposited on the surface of the CaCO₃ microparticles. In the second step, the CaCO₃ core was removed by complexation with EDTA. Therefore, the coated CaCO₃ particles were shaken for 30 min with 1 mL of EDTA-solution (0.2 M, pH 7.5) followed by centrifugation and redispersion in 1 mL of fresh EDTA-solution. This procedure was repeated four times to assure complete removal of the CaCO₃ core as previously reported by Volodkin et al.[18,19] Finally, the obtained hollow microcapsules filled with FITC-dextran were washed four times with water.

Electrophoretic Mobility

The electrophoretic mobility of the AuNP was measured using a Malvern Zetasizer 2000 (Malvern Instruments). The \( \zeta \)-potential was calculated from the electrophoretic mobility \( \mu \) using the Smoluchowski relation: \( \zeta = \mu / \eta / \varepsilon \), where \( \eta \) and \( \varepsilon \) are the viscosity and permittivity of the solvent, respectively.

Scanning Electron Microscopy (SEM)

A drop of particle or capsule suspension was deposited onto a silicon wafer and dried under nitrogen stream followed by sputtering with gold. SEM images were recorded with a Quanta 200 FEG FEI scanning electron microscope operated at an acceleration voltage of 5 kV.

Transmission Electron Microscopy (TEM)

A drop of AuNP or capsule suspension was deposited and dried onto a Cu-grid modified with amorphous carbon. TEM images were recorded with a CM-200 FEG Philips transmission electron microscope operated at an acceleration voltage of 120 kV.

Confocal Microscopy

Confocal microscopy and transmission light microscopy images were recorded with a BioRad MRC 1024 confocal system. An inverted microscope (Eclipse TE300D, Nikon) equipped with a 60× water-immersion objective lens was used.

Confocal Raman Microscopy

A RamanRxn1 spectrometer (Kaiser Optical Systems), equipped with an air-cooled charge coupled device (CCD) Detector (back-illuminated deep depletion design) was used in combination with a Leica DM LP Raman microprobe equipped with a 100× objective. The laser wavelength during the experiments was the 785 nm line from a 785 nm Invictus NIR diode laser. All spectra were recorded at a laser power of 400 mW.

IR Laser Setup

The collimated laser beam of a CW laser diode at 830 nm with optical power up to 80 mW was focused onto the sample through a microscope 100× objective. A more detailed description of the setup can be found in ref.[21] Illumination was performed by a 150 W white light sources and a fluorescence lamp. Images were recorded by a CCD camera connected to a computer. The solution containing the capsules was deposited onto a microscope slide under the microscope objective.

Results and Discussion

Gold nanoparticles (AuNP) were synthesized following the method reported by Kimura et al.[22] by reduction of hydrogen tetrachloroaureate trihydrate (HAuCl₄ 3H₂O) with NaBH₄ in methanol. MSA was used to cap the formed gold nanoparticles with a monolayer of carboxyl groups. The \( \zeta \)-potential of the AuNP was measured to be \( -31.9 \pm 7.6 \) mV, indicating that the carboxyl groups of the MSA provide the AuNP with a negative surface charge. TEM [Figure 1(A)] revealed that the AuNP are in a non-aggregated state and have a size distribution ranging from approximately 1 to 5 nm. The UV-vis spectrum of the AuNP in concentrated solution is shown in Figure 1(B), exhibiting the characteristic surface plasmon resonance absorption peak around 560 nm.[23]

As the AuNP exhibit a negative surface charge due to the carboxyl groups of the MSA, they should be suitable for the fabrication of multilayered films using the LbL technique. Poly(allylamine hydrochloride) (PAH), which is a positively
charged polyelectrolyte (at neutral pH) by virtue of protonation of its amino groups, was used as polycation. As AuNP absorb light in the UV-vis region, UV-vis spectrophotometry can be used to monitor the multilayer build-up of the AuNP in combination with PAH onto a quartz slide. As shown in Figure 1(C) the absorbance of the film increases after every deposited AuNP/PAH bilayer, indicating that multilayer build-up takes place. The absorbance of the deposited film does not show the plasmon peak at 560 nm due to the fact that the optical absorbance of extremely thin films is hardly detectable with conventional UV-vis measurements. Figure 1(D) shows the increase in absorbance at 450 nm, apparently a linear multilayer build-up is observed during the deposition of the six AuNP/PAH bilayers. The multilayer build-up was stopped after six bilayers as this number would be largely sufficient with respect to the fabrication of AuNP/PAH capsules.

Multilayered capsules from AuNP/PAH were fabricated using calcium carbonate (CaCO3) microparticles as sacrificial templates. FITC-dextran filled CaCO3 particles were synthesized by reacting calcium chloride and sodium carbonate solutions in the presence of FITC-dextran (2 000 kDa). Immediately after mixing of the components the precipitation of 4 μm-sized CaCO3 microparticles, filled with FITC-dextran, takes place. These particles served as templates for the alternate adsorption of AuNP and PAH. Initially, the CaCO3 particles are slightly negatively charged, but due to their high porosity it is possible to absorb almost any desired species. In total four AuNP/PAH bilayers were deposited onto the surface of the CaCO3 microparticles. After the deposition of the (AuNP/PAH)4 coating, the CaCO3 microparticles were dissolved in EDTA solution which complexes the Ca2+ ions. After several washing steps with water, removing the dissolved ions, hollow capsules filled with a FITC-dextran solution are obtained. Figure 2 shows SEM images of FITC-dextran filled CaCO3 microparticles (A) before and (B) after coating with four AuNP/PAH bilayers. It can be clearly observed from the SEM images that the uncoated CaCO3
microparticles exhibit a smooth surface, whereas after coating the CaCO₃ microparticles exhibit a rather rough surface. Figure 2(C) shows SEM images of hollow capsules obtained after dissolution of the CaCO₃ core. Collapsed (due to the drying upon preparation of the sample prior to SEM imaging) capsules with a rough surface morphology are observed. Such rough surface is contrary to the rather smooth surface of capsules fabricated from solely polymeric species (i.e., polyelectrolytes).[24] Figure 2(D) shows a confocal microscopy image of the capsules depicting homogeneous filling of the capsules with FITC-dextrans. Figure 2(E) and 2(F) show TEM images of a (AuNP/PAH)₄ capsule at low [Figure 2(E)] and high [Figure 2(F)] magnification. A very dense network of AuNP which are “glued” together by electrostatic interactions with the PAH is observed. On the high magnification image [Figure 2(F)] one can clearly distinguish the individual AuNP which are closely packed to each other. These TEM images of the (AuNP/PAH)₄ capsules are substantially different from those reported by Skirtach et al.[16] where only one layer of AuNP was intercalated within the capsule wall. TEM images from such capsules show AuNP which are sparsely spread within the capsule wall, whereas the AuNP in the (AuNP/PAH)₄ capsules reported in this paper form a tight network.

It is known that PAH is a weak polyelectrolyte, meaning that its charge density is strongly dependent on the pH of the environment.[25] Whereas the amino groups of the PAH are protonated, and thus positively charged, at a pH below 10.7 (the pKₐ of PAH when it is complexed to a polyanion was determined to be 10.7 by Petrov et al.[24]), they become uncharged when the pH of the surrounding medium is above 10.7. In literature several papers have been published describing the swelling and dissolution behavior of polyelectrolyte multilayers from weak polyelectrolytes.[26–28] The interactions between the successive layers in the (AuNP/PAH)₄ capsules reported in our present paper are also based on the interactions between a weak polyacid, as the AuNP have carboxyl acid groups on their surface, and a weak polybase (i.e., PAH). Therefore, we were interested to know whether our (AuNP/PAH)₄ capsules exhibited a dual responsive behavior both at low and high pH. Figure 3 shows transmission light microscopy images of the (AuNP/PAH)₄ capsules before and after addition of 0.5 M HCl respectively 0.5 M NaOH. The capsules swell approximately twice in diameter and then gradually dissolve leading to the release of the encapsulated material. At high pH...
pH the amino groups of the PAH become uncharged and no longer complex with carboxyl groups of the AuNP, leading to a loosening of the multilayer structure. As the negative charges of the carboxyl groups of the AuNP are no longer compensated by the amino groups of the PAH repulsion between the carboxyl groups occurs. Due to this repulsion, the capsules swell until they completely dissolve. At low pH, the inverse phenomenon occurs as the carboxyl groups of the AuNP become uncharged and no longer repel the positively charged amino groups of the PAH, again leading to a swelling followed by the dissolution of the capsules. Furthermore, we were interested to examine whether it was possible to covalently cross-link the multilayer membrane and thus make the capsules invariant to pH changes in their environment. However, cross-linking between nanoparticles and polyelectrolytes within the shell of multilayered capsules has been reported; it has already been reported that multilayered capsules consisting of the weak polyelectrolytes poly(methacrylic acid) and PAH could be cross-linked and retain their properties over the whole pH range. Therefore, an identical procedure was used to react the carboxyl groups of the AuNP and the amino groups of the PAH using conventional carbodiimide chemistry resulting in the formation of amide bonds. When the cross-linked capsules were placed in solutions of 0.5 M HCl or NaOH they do not swell and remain intact. Moreover, no significant changes in morphology or fluorescence take place indicating that the cross-linking reaction prevents the escape of the encapsulated species. Apparently the cross-linked hybrid nanoparticle/polyelectrolyte layers are still able to encapsulate macromolecules even when the electrostatic interactions are no longer contributing to the integrity of the capsules (data not shown).

It has been reported that polyelectrolyte capsules containing AuNP in their shell can strongly be affected when irradiated with an IR laser, resulting in the destructing of the capsules. The polyelectrolyte embedded AuNP absorb IR-light causing in a local heating of the nanoparticles finally resulting in the disruption of the polyelectrolyte membrane. As IR-light is not harmful to living tissue, the use of IR light is an attractive method for opening polyelectrolyte capsules for biomedical applications. The IR-responsive properties of our (AuNP/PAH)$_4$ capsules were assessed by irradiating them with a focused laser beam of 30 mW. Figure 4 shows the impact of the IR irradiation on a cluster of (AuNP/PAH)$_4$ capsules. Shortly after exposure of the capsules cluster to the IR laser, the whole cluster explodes and only debris of broken capsules can be observed. Also, the bright color of the encapsulated FITC-dextran inside the (AuNP/PAH)$_4$ capsules has disappeared. The advantages of this type of capsules, fabricated from CaCO$_3$ microparticles as sacrificial template, compared to previously reported IR-sensitive capsules are three-fold:

1. Whereas the dissolution of melamine formaldehyde (MF) or polystyrene (PS) microparticles require the use of harsh conditions, such as extreme low pH or organic solvents to dissolve the core template, CaCO$_3$ can be dissolved at physiologic pH using EDTA as complexation agent for the Ca$^{2+}$.

2. The use of MF or PS microparticles are sacrificial core template suffers from remaining MF or PS oligomers/polymer inside the capsule wall, making the capsules unsuited as drug delivery system. The dissolution products of CaCO$_3$ are small ions which can easily permeate through the multilayer wall; moreover, they are non-toxic.

3. The use of MF or PS microparticles as sacrificial core template require additional steps to reversibly open and close the capsules’ wall in order to obtain filled capsules. CaCO$_3$ microparticles can easily be filled with macromolecules by coprecipitation of the macromolecules during the fabrication of the CaCO$_3$. After LbL coating and dissolution of the CaCO$_3$ one obtains directly filled capsules.

4. The low cost and ease of production allows the large quantity fabrication of capsules which would be required for clinical testing.

![Figure 4. Fluorescence microscopy images of a cluster of FITC-dextran (bright color)-filled (AuNP/PAH)$_4$ capsules upon irradiation with IR laser light. The time interval between the successive images is 2 s.](image-url)
Therefore, for the applications as drug delivery system we envision, the CaCO₃ templated capsules are ideally suited.

We wished to know whether the (AuNP/PAH)₄ capsules could be detected with Raman microspectroscopy. Raman spectroscopy is based on the shift in wave number of monochromatic IR light upon inelastic scattering by a substance.[33] Raman microspectroscopy allows one to collect data from a well-defined area with microscopic dimensions which offers the possibility for mapping an area based on the interactions of the chemical compounds with IR light in this area. So, the capsules were dried onto a quartz slide and put under the confocal Raman microscope. Figure 5(A) shows a Raman mapping image of a (AuNP/PAH)₄ capsule while Figure 5(B) shows the interpolated image. The Raman spectra inside and outside the capsule are shown in Figure 5(C). This spectrum of the (AuNP/PAH)₄ capsules was also found to be similar to the spectrum of the free AuNP suspended in water, proving that the measured Raman shifts are unique due to the AuNP. Therefore, sensitivity of the (AuNP/PAH)₄ capsules to IR light can not only be used for release purpose but can also be used for spectroscopic purpose, which might lead to application of these capsule in the diagnostic field.

**Conclusion**

In conclusion, we are the first to report on the fabrication of hybrid nanoparticle/polyelectrolyte capsules by multilayer formation of carboxylic acid functionalized AuNP and PAH. Several microscopic techniques were used to characterize the capsules. As both AuNP and PAH contained weak acid and weak amino groups, respectively, it was possible to deconstruct the capsules at low and high pH. Furthermore, it was shown that covalent cross-linking of the capsule wall was an effective method to render the capsules invariant to changes in pH. The AuNP incorporated in the capsules’ wall rendered the capsules responsive to IR-light, which can be used at high intensity to destroy the capsules and at low intensity to perform imaging. The use of CaCO₃ as sacrificial template for capsule fabrication makes the capsules ideally suited for applications in the drug delivery field. In our ongoing research, the capsules will be used for transdermal[34] activated drug release.

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